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STUDIES ON THE SV40-LIKE PAPOVAVIRUS SV40-GBM. III. PROPAGATION AT LOW MULTIPLICITIES OF INFECTION IN VARIOUS HUMAN CELL LINES

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Summary. — At low multiplicity several human cell lines supported the lytic infection with SV40-GBM better than that with the wild type SV40. The efficiency of viral DNA replication differed in the cell lines used suggesting that specific host cell factors may determine the rate of viral DNA synthesis. Furthermore, the emergence of different DNA defects during propagation of the virus indicates that host cell factors in question might also influence the composition of the viral DNA population.

Key words: SV40 strain differences; DNA synthesis; host cell factors

Introduction

As already described we isolated an SV40-like papovavirus designated SV40-GBM from the brain of a patient with glioblastoma multiforme (Scherneck *et al.*, 1979; Geissler *et al.*, 1980*a*) by fusion of the in vitro cultured M27 glioblastoma cells with CV-1 monkey cells permissive for SV40 replication. SV40-GBM is similar to SV40 wild-type (WT) with respect of morphology, size, DNA characteristics and antigenicity (Scherneck *et al.*, 1979; Geissler *et al.*, 1980*b*). Like SV40-WT it does not agglutinate human 0 erythrocytes. SV40-GBM differs slightly, however, from SV40-WT in several respects: (1) digestion of SV40-GBM DNA with HindIII restriction endonucleases revealed minor differences compared with the SV40 DNA (Scherneck *et al.*, 1979); (2) the virus can be replicated and exhibit cytopathic effect (CPE) in both CV-1 monkey cells and primary foetal human cells (fibroblasts and glial cells). CPE in human cells is not so pronounced as in CV-1 monkey cells, but about 50—60% of the cells showed intranuclear fluorescence when stained with SV40 antibodies on day 10 post-infection (p. i.) (Scherneck *et al.*, 1979; Geissler *et al.*, 1980*a*, 1984); (3) already after three passages of SV40-GBM in CV-1 cells at low multiplicities (MOI $\leq$ 10) variant DNA molecules arise (SV40-GBM3) which fall into two main fractions, SV40-GBM3-H and SV40-GBM3-L. Both viral DNA fractions have been cloned and characterized by cleavage with restriction endonucleases and heteroduplex analysis. One of them, GBM-H, was indistinguishable

from the DNA of the original GBM isolate. The DNA molecules of the smaller variant, GBM-L, were about 19 % shorter and showed different alterations in the late genome region (Zimmermann *et al.*, 1983; Krause *et al.*, 1983). In the present study we have surveyed a number of human cell types for their ability to support the growth and defective production of SV40 and SV40-GBM, respectively.

Materials and Methods

Cells and virus. All cell lines used are listed in Table 1. They were propagated in Eagle's minimum essential medium (MEM) or RPMI-medium with 10% bovine or foetal bovine serum (SIFIN, Berlin). The large plaque strain of SV40 and SV40-GBM have been described previously (Böttger *et al.*, 1976; Scherneck *et al.*, 1979). The SV40-DAR virus (Weiner *et al.*, 1972) was obtained from Dr. K. K. Takemoto (Bethesda, U.S.A.). The generation of plaque purified SV40 and SV40-GBM, respectively, has also been described (Scherneck *et al.*, 1979).

Plaque test. The plaque test was performed on CV-1 cells using a 20 mmol/l TES-buffer system as described by Itakagi and Kimura (1974).

Extraction, purification and analysis of viral DNAs. Cells were infected with the virus at low multiplicities (MOI ~ 10). Viral DNA was extracted and purified from infected cells on day 7 p.i. by the method of Hirt (1967). Viral DNAs were then separated by electrophoresis through 1.2% (w/v) agarose gel slabs, as previously described (Krause *et al.*, 1983). Radiolabelling, blotting and hybridization of the DNAs followed the original protocols of Southern (1975), Rigby *et al.* (1977) and Wahl *et al.* (1979).

Results

Infection of various human cell lines with SV40-GBM, SV40-DAR and SV40-WI

Subconfluent monolayers of human cells grown in small 25 ml glass bottles (containing about 1×10^6 cells) were infected with SV40-GBM, SV40-DAR and SV40-WT at MOI of 10 PFU/cell. The infection was initiated with plaque-purified viruses which were propagated at MOI ~ 10^{-4} PFU/cell on CV-1 cells. SV40- and SV40-DAR virus stocks were essentially free of defective particles, the SV40-GBM stock contained a low level of defectives (Zimmermann *et al.*, 1983). The presence of defective viral genomes was assayed by agarose gel electrophoresis of Hirt supernatant DNAs which were collected in the low multiplicity passage.

Morphological changes observed for the various cell lines after infection with SV40-WT, SV40-GBM and SV40-DAR were quite different; infection of Fi294-, HeLa-,RH30-and Matu-cells resulted in destruction of about 20 % of the cells but this destruction was not initially observed usually until about 4 weeks p. i. On the other hand, infection of A172-, A549- and RD3--cells resulted in destruction of about 50-60 % of cells within approximately 3 weeks p. i., whereas a comparable CPE could be observed after infection of Fl 183 cells with SV40-GBM but not with SV40 and SV40-DAR. In general, infection of human cells initially resulted in cytopathic changes characterized by cell rounding and pyknosis. In accordance to Miyamura *et al.* (1983), cytoplasmic vacuolization which is frequently seen in African green monkey cells (CV-1) infected with SV40 and SV40-GBM, was seldom

observed. By 10 days most of the CV-1 cells were completely destroyed after infection with all 3 viruses used. To compare viral growth in both, human and CV-1 cells, the assay was performed 7 days p. i.

Replication of SV40-GBM, SV40-DAR and SV40-WT in various human cell lines

As already described, SV40-GBM replicates better in primary human fibroblasts and primary human glial cells than SV40-WT and SV40-DAR (Geissler *et al.*, 1980*b*). In the present study we have examined 8 different human cell lines for their ability to support replication of SV40-GBM. The cells included in the study are described in Table 1. Viral replication was first assayed by agarose gel electrophoresis of Hirt supernatant DNA (Hirt, 1967). DNA species migrating ahead of the WT band were scored as defective. As it is shown in Figs 1A and B, the human cell lines differed in their ability to support viral growth as follows: (1) Whereas RD3-, A549- and Fl 183 cells supported efficient replication of SV40 and SV40-GBM, other cell lines like HeLa, RH30 and Fi294 were much less sensitive to SV40 infection as compared to SV40 DNA grown in CV-1 monkey cells. (2) most of the human cell lines tested supported replication of SV40-GBM better than SV40 and SV40-DAR (Fl 183, Matu, RH30, Fi294, HeLa). On the other hand in RD3- and A594-cells, the titres of viral DNA obtained were roughly equivalent to those obtained in African green monkey cell lines. However, the human glioblastoma cell line, A172, supported lytic infection by SV40-WT to high titre much better than SV40-GBM and SV40--DAR, respectively.

In order to provide evidence that virus is produced in the different human cell lines infected with SV40, SV40-GBM and SV40-DAR, respectively, viral lysates were assayed for infectivity in CV-1 cells by plaque assay. Virus production occurred in all cell lines used (Table 1). In most of the human cell lines SV40-GBM was produced in higher titre than SV40. However, the differences in the total yield between SV40-GBM and SV40-WT were not very pronounced, being 2—5 times higher for SV40-GBM than for SV40--WT. In this respect the efficient replication of SV40-GBM DNA in some human cell lines did not result in the production of high titres of infectious virus. In A172 cells SV40-WT showed a higher titre than SV40-GBM and SV40-DAR. In contrast to these results in CV-1 monkey cells the total yield of SV40-WT, SV40-GBM and SV40-DAR, respectively, was by about one order magnitude higher than in human cells.

In an earlier report we showed that SV40-GBM is highly unstable and gives rise to variant DNA molecules after only few passages in CV-1 cells at low (about 10) MOI. These DNA fractions have been cloned and characterized by cleavage with restriction endonucleases and heteroduplex analysis (Zimmermann *et al.*, 1983). Our present study dealing with replication of SV40-GBM, SV40-DAR and SV40-WT, respectively, in different human cell lines indicate that defective viral genomes emerge in several human cell lines. The presence of defective viral DNA molecules was assayed by agarose gel electrophoresis of Hirt supernatant DNA. As shown in Figs 1—2,

Table 1. Replication of SV40-WT, SV40-GBM and SV40-DAR in various cell lines

Cells	Derivation	Source	Virus[a]					
			SV40		SV40-GBM		SV40-DAR	
			Growth PFU/ml ($\times 10^5$)	Defectives[b]	Growth PFU/ml ($\times 10^5$)	Defectives	Growth PFU/ml ($\times 10^5$)	Defectives
Fi 294	human foetal fibroblasts	IAV, Berlin, GDR	7	−	12	+	N. T.	N. T.[c]
RH 30	human rhabdomyosarcoma	IAV, Berlin, GDR	2	−	6	−	N. T.	N. T.
A 172	human glioblastoma	F. J. O'Neill, Salt Lake City, USA	30	+	10	+	10	+
FI 183	human amnion	IAV, Berlin, GDR	5	−	25	±	4	−
RD 3	human rhabdomyosarcoma	IAV, Berlin, GDR	15	−	20	+	N. T.	N. T.
HeLa	human cervical carcinoma	ZIK, Berlin, GDR	10	−	20	±	N. T.	N. T.
A 549	human lung carcinoma	L. Grosjean, Villejuif, France	30	+	40	±	N. T.	N. T.
Matu	human mammary tumor	ZIK, Berlin, GDR	3	−	15	±	10	−
CV-1	African green monkey kidney	Flow-Laboratories	180	−	130	+	100	−

[a] The data shown for each cell line are representative of at least three independently performed experiments.
[b] The presence of defective viral genomes was assayed by agarose gel electrophoresis of Hirt supernatant DNAs.
[c] Not tested.

and Table 1, SV40-GBM defectives arose in nearly all of the human cells used for virus propagation. In contrast to this observation, SV40-WT defectives emerged only in A549 and A172 cells. The latter finding is consistent with reports of O'Neill and Carroll (1978) showing the apperance of defective SV40 DNA molecules following infection of human glioblastoma cells. The different pattern of defectives in the cell lines used indicate that they were generated in the cell line and not selected from an undetected fraction in the original inoculum.

Discussion

Human cells are generally semipermissive hosts for SV40-WT replication (Tooze, 1980). However, at least two among the numerous human cell lines tested appear to be highly susceptible to SV40, namely the human glioblastoma cell line. A172 (O'Neill, 1976) and a cell line established from skin biopsies of patients with Wiscott-Aldrich Syndrome (Miyamura *et al.*, 1983). The basis for the unusual susceptibility of these cell lines remains to be determined. On the other hand, we had isolated an SV40-like papovavirus, designated SV40-GBM, which replicated better than SV40-WT in primary human fibroblasts and primary glial cells (Geissler *et al.* 1980*a*). In this respect the SV40-GBM resembles another SV40-like virus, SV40-DAR, which was isolated from the brain of a patient with PML (Weiner *et al.*, 1972). The results presented here support an extend our earlier reports that SV40-GBM can be better replicated in several human cell lines than SV40--WT. However, these lines differ in their ability to support efficient viral replication and, as mentioned above, the A127 cell line support lytic infection by SV40-WT to high titre much better than SV40-GBM and SV40-DAR, respectively. The reasons for these differences remain unclear. They also cannot be explained only by changes of specific regions on the viral genome which are thought to be important in determining the host range of the virus (Laimins *et al.*, 1982). Our results rather indicate that specific host cell factors may play a role in determining the rate of viral DNA replication and the composition of the viral DNA population. The latter statement is supported by the finding that in spite of the relatively low MOI used and the low virus passage number, defective virus particles emerged and the pattern of DNA defects was different. Similar results and conclusion have been published by Norkin *et al.* (1981) on the effect of the host cells on the generation of defective SV40 during undiluted serial passages in monkey CV-1 GMK- and LLC-MK_2 cells and O'Neill and Carroll (1982) for propagation of SV40 and BKV after low MOI in different human cell lines. The emergence of different kinds of defectives may also influence the titre of infectious virus which did not correlate with the amount of viral DNA produced in every human cell and CV-1 monkey cells, respectively. However, a more detailed analysis is necessary to clear up this phenomenon.

References

Böttger, M., Scherneck, S., and Fenske, H. (1976): A sedimentation study of the interaction of supercoiled SV40 DNA with H1 histone. *Nucleic Acids Res.* **3**, 419—429.

Geissler, E., Scherneck, S., Waehlte, H., Zimmermann, W., Luebbe, L., Krause, H., Theile, M., Herold, H.-J., Rudolph, M., Weickmann, F., and Nisch, G. (1980*a*): Further studies on the relationship of SV40-like viruses to human tumors, pp. 343—356. In M. Essex, G. Todaro and H. zur Hausen (Eds): *Viruses in Naturally Occurring Cancers*, Book B, CSH Conference on Cell Proliferation, Vol. 7, Cold Spring Harbor Laboratory, Cold Spring Harbor.

Geissler, E., Scherneck, S., Theile, M., Herold, H.-J., Staneczek, W., Zimmermann, W., Krause, H., Prokoph, H., Vogel, F., and Platzer, M. (1980*b*): Studies on the mutagenic and transforming activities of SV40-like viruses, pp. 43—55. In P. A. Bachmann (Ed.): *Leukaemias, Lymphomas and Papillomas: Comparative Aspects*, Taylor and Francis, London.

Geissler, E., Scherneck, S., Prokoph, H., Zimmermann, W., and Staneczek, W. (1984): SV40 in human brain tumors: risk factor or passenger? pp. 265—279. In E. Beth and G. Giraldo (Eds): *The Role of Viruses in Human Cancer*, Vol. II, Elsevier, North-Holland, Amsterdam.

Hirt, B. (1967): Selective extraction of polyoma DNA from infected mouse cell cultures. *J. molec. Biol.* **26**, 365—369.

Itakagi, A., and Kimura, G. (1974): Tes and Hepes buffers in mammalian cell cultures and viral studies: problem of carbon dioxide requirement. *Exp. Cell Res.* **83**, 351—361.

Krause, H., Garajev, M. M., Geissler, E., Platzer, M., Scherneck, S., and Zimmermann, W. (1983): Studies on the SV40 like papovavirus SV40-GBM. II. Molecular cloning in *Escherichia coli* of variant DNA molecules from glioblastoma-derived virus. *Gene* **26**, 19—24.

Laimins, L. A., Khoury, G., Gorman, C., Howard, B., and Gruss, P. (1982): Host specific activation of transcription by tandem repeats from Simian virus 40 and Moloney murine sarcoma virus. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6453—6457.

Miyamura, T., Yoshiika, K., and Takemoto, K. K. (1983): High titer replication in human fibroblast cell lines derived from patients with Wiscott-Aldrich-Syndrome. *Virology* **129**, 479—483.

Norkin, L. C., Wojcik, J. B., and Goguen, C. A. (1981): Effect of the host cell on the generation of defective Simian virus 40 during undiluted serial passages and persistent infection. *Virology* **108**, 525—530.

O'Neill, F. J. (1976): Propagation of Simian virus 40 in a human cell line. *Virology* **72**, 287—289.

O'Neill, F. J., and Carroll, D. (1978): Appearance of defective Simian virus 40 following infection of cultured human glioblastoma cells. *Virology* **87**, 109—119.

Rigby, P. W., Dickman, M., Rhodes, C., and Berg, P. (1977): Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. molec. Biol.* **113**, 237 —251.

Scherneck, S., Rudolph, M., Geissler, E., Vogel, F., Luebbe, L., Waehlte, H., Nisch, G., Weickmann, F., and Zimmermann, W. (1979): Isolation of a SV40-like papovavirus from a human glioblastoma. *Int. J. Cancer* **24**, 523—531.

Southern, E. (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. molec. Biol.* **98**, 503—517.

Wahl, G. M., Stern, M., and Stark, G. R. (1979): Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextrane sulfate. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683—3687.

Weiner, L. P., Herndon, R. M., Narayan, O., Johnson, R., Shah, K., Rubinstein, L. J., Preziosi, T.J., and Conley, F. K. (1972): Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. *N. Engl. J. Med.* **286**, 385—390.

Zimmermann, W., Platzer, M., Scherneck, S., Vogel, E., Krause, H., Prokoph, H., and Geissler, E. (1983): Studies on the SV40 like papovavirus SV40-GMB. I. Genomic analysis by restriction endonucleases and electron microscopy after propagation in CV-I monkey cells. *J. gen. Virol.* **64**, 733—737.

Explanation to Figures (Plates XI—XII):

Figs 1—2: Analysis of electrophoretic separated Hirt-supernatants of various human cell lines after infection with SV40-WT, SV40-GBM and SV40-DAR virions, respectively. The DNAs were electrophoresed on a 1.2% agarose gel, transferred to a nitrocellulose filter and hybridized to ^{32}P-labelled SV40 DNA under stringent conditions (T_m — 28 °C). The filters were autoradiographed for 2 days. In control experiments Hirt supernatants of CV-1 monkey cells infected with SV40-WT were analysed.

1 — Lanes A, K, N: CV-1 monkey cells infected with SV40-WT
Lanes B, E, H, L: human cells infected with SV40-WT
Lanes C, F, I, M: human cells infected with SV40-GBM
Lanes D, G, J: human cells infected with SV40-DAR
2 — Lanes C, F, I, L: CV-1 monkey cells infected with SV40-WT
Lanes A, D, G, J: human cells infected with SV40-WT
Lanes B, E, H, K: human cells infected with SV40-GBM